FORMATION OF A COMPLEX BETWEEN 23 S RNA, 5 S RNA AND PROTEINS FROM *ESCHERICHIA COLI* 50 S RIBOSOMAL SUBUNITS

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1. Introduction

It has been previously shown that the core and split fractions derived from *Escherichia coli* 50 S ribosomal subunits by 2 M LiCl treatment can be used to study the binding of 5 S RNA to reconstituted particles [1, 2]. Although the reconstituted particles have no biological activity, the binding of 5 S RNA proved to be quite specific [2] and this system can therefore be used to explore the recognition of 5 S RNA by other ribosomal components. It was possible to show as an example that denatured 5 S RNA cannot be incorporated into reconstituted particles [3], while molecules with a single hidden T_1 RNase-break at position G 41 are fully competitive with intact molecules for incorporation [4].

Ribosomal proteins present in the split fraction are necessary for the formation of a stable ribonucleoprotein complex containing both 23 S RNA and 5 S RNA in stoichiometric amounts [2]. As a step towards the definition of the minimal protein requirement for the building of such a complex, we have now studied the formation of complexes starting from phenol-deproteinized 23 S RNA, purified ^{32}P-5 S RNA and some of the proteins from the 2 M LiCl split. A preliminary characterization of the proteins involved in the association between 23 S RNA and 5 S RNA has been achieved. The results lead to the conclusion that only a few of the 2 M LiCl split proteins are directly involved in the binding of 5 S RNA to the 50 S ribosomal subunit.

2. Experimental

Ribosomes and ribosomal subunits were prepared from *Escherichia coli* RNase I^-_{10} [5] as previously described [6]. 2 M LiCl cores and splits were prepared from isolated subunits according to Marcot-Queiroz and Monier [1].

DEAE cellulose chromatography of the 2 M LiCl split was performed on Whatman DE 32 DEAE-cellulose according to Nomura and Erdmann [7]. After collecting the fraction which is not adsorbed at a concentration of 0.2 M KCl (fraction I), four fractions were obtained by stepwise elution at 0.3 M KCl (II), 0.5 M KCl (III), 0.7 M KCl (IV) and 1.0 M KCl (V). Fraction III contained all the 5 S RNA present in the 2 M LiCl split. Preliminary tests on fractions I to V, used either one at a time or in various combinations, demonstrated that fraction I was the only one which was absolutely necessary to form a stable 23 S RNA– 5 S RNA– protein complex. Fraction I was therefore used in the experiments described here.

^{32}P-5 S RNA was prepared as described by Monier and Feunteun [8] and was the generous gift of Dr. B.R. Jordan. 23 S RNA and 16 S RNA were isolated by sucrose gradient sedimentation from total ribosomal RNA prepared by sodium dodecyl sulfate-phenol deproteinization according to published methods [9]. The deproteinization was repeated three times to ensure as complete a removal of proteins as possible.

Prior to experiments, RNA samples and fraction I proteins were extensively dialyzed against the reconstitution buffer of Nomura and Erdmann [7] (10 mM Tris-HCl, pH 7.6, 300 mM KCl, 20 mM $MgCl_2$, 6 mM β-mercaptoethanol). 23 S RNA, ^{32}P-5 S RNA and

fraction I proteins were mixed in appropriate amounts and incubated at 37° for 30 min unless otherwise stated and complex formation was measured by filtration on nitrocellulose filters (Millipore, type HA-0.45 μm) or by sucrose gradient sedimentation. For filtration experiments, the amount of 23 S RNA was kept below 2 A_{260} units per assay and the final incubation volume was 0.1 ml. After incubation, the reaction mixture was quickly diluted with 3 ml of cold reconstitution buffer and filtered. The filters were washed three times with 3 ml of cold buffer and dried before counting. For sucrose gradient analysis, the amount of 23 S RNA was increased to about 3 A_{260} units and the final incubation volume was 0.2 ml. After incubation, the reaction mixture was quickly cooled to 0° and layered on a 5–20% sucrose gradient in the appropriate buffer and sedimented in a Spinco SW 27 rotor at 25,000 rpm for 18 hr at a temperature of 1°. The tube content was fractionated and processed as previously described [9].

Electrophoresis of proteins was performed according to Weber and Osborn [10] on 0.6 X 7.5 cm 10% acrylamide gels. The gels were stained with aniline blue-black and electrophoretically destained in 7% (v/v) acetic acid. Measurement of protein amounts was made according to Lowry et al. [11] using bovine serum albumin as a standard.

Radioactivity was measured in a Tri-Carb Packard spectrometer in Bray's scintillation fluid [12].

3. Results

When increasing amounts of fraction I protein were incubated with constant amounts of 23 S RNA and ^{32}P-5 S RNA, the radioactivity retained on nitrocellulose filters linearly increased from a background level of 10 cpm to a maximum of 1775 cpm. In fig. 1, the results are expressed in terms of the molar ratio of the ^{32}P-5 S RNA retained on the filter to the 23 S RNA present in the incubation mixture. At saturation, the ratio reaches 0.8, i.e. a value close to the molar ratio of 5 S RNA to 23 S RNA in the 50 S subunit [13] or in reconstituted particles [2].

Control experiments were made under similar conditions with the following variations:
(1) 23 S RNA was replaced by 16 S RNA;
(2) ^{32}P-5 S RNA was replaced by denatured 5 S RNA (B form) [3];

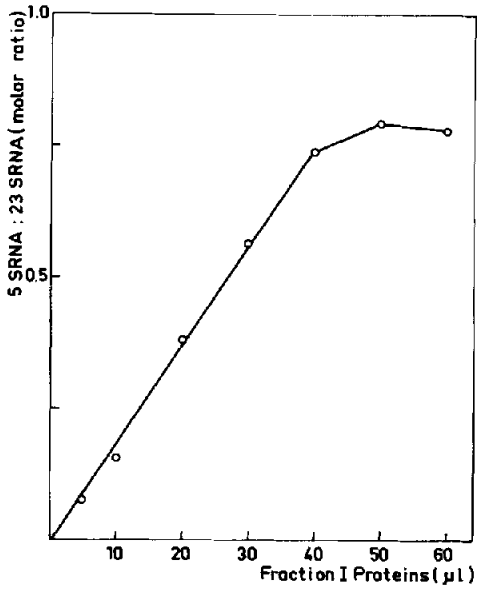

Fig. 1. Binding of ^{32}P-5 S RNA to nitrocellulose filters in the presence of 23 S RNA and of fraction I proteins. Each incubation mixture contained 1.2 A_{260} unit of 23 S RNA and 0.12 A_{260} unit of ^{32}P-5 S RNA (6550 cpm). The concentration of the fraction I protein solution was 1.2 mg/ml.

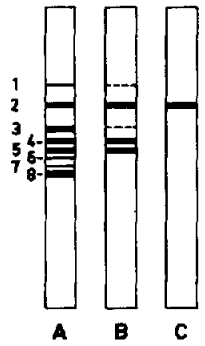

Fig. 2. Electrophoretic analysis of proteins in fraction I and in ribonucleoprotein complexes isolated on sucrose gradients. A: proteins in fraction I. B and C: the ribonucleoprotein complexes were prepared by incubating at 30° for 30 min in a final volume of 0.4 ml, 8.6 A_{260} units of 23 S RNA, 0.48 A_{260} units of 5 S RNA and 460 μg of fraction I proteins (B). In C, 5 S RNA was omitted from the incubation mixture. After sedimentation on 5–20% sucrose gradients prepared in reconstitution buffer in the Spinco SW 27 rotor at 26 000 rpm for 16 hr, material present in the 23 S peak was collected and recovered by ethanol precipitation in the presence of 5.7 A_{260} units of carrier 23 S RNA. Equal numbers of A_{260} units were used for the analyses shown in B and C.

Table 1
Stability of the ribonucleoprotein complex on sucrose gradients.

	Concentrations in the gradients (mM)		^{32}P-5 S RNA bound to 23 S RNA (cpm)
	KCl	$MgCl_2$	
Experiment 1	300	10	15 800
	300	5	16 300
	300	2	0
Experiment 2	30	5	7 690
	30	1	7 770
	30	0.1	8 200

In experiments 1 and 2, 3.1 A_{260} units of 23 S RNA, 0.19 A_{260} units of ^{32}P-5 S RNA and 190 μg of fraction I proteins were incubated together. The radioactivity of ^{32}P-5 S RNA was 30,000 cpm in experiment 1 and 15,000 cpm in experiment 2.

(3) ^{32}P-5 S RNA was replaced by ^{32}P-tRNA;

(4) The fraction I proteins from the 50 S subunit split were replaced either by the total 30 S subunit split or by the fraction of the 30 S subunit split which did not adsorb to DEAE cellulose in 0.2 M KCl. No significant radioactivities were retained on the filters in these four control experiments. It can therefore be concluded that the complex which was retained on the filters in the first experiment was specific of 5 S RNA, 23 S RNA and 50 S subunit proteins. Its formation had the same requirement with respect to 5 S RNA conformation as 5 S RNA binding to reconstituted particles [3].

In order to characterize the complex further and especially to determine its stability under various ionic conditions, reaction mixtures containing saturating amounts of fraction I proteins were layered on sucrose gradients prepared in the presence of different concentrations of KCl and $MgCl_2$. The radioactivity sedimenting in association with 23 S RNA was measured. When the KCl concentration in the gradient was kept at 300 mM, as in the reconstitution buffer, no decrease in the radioactivity bound to 23 S RNA was observed upon reduction of the Mg^{2+} concentration to 5 mM. However below 2.5 mM Mg^{2+}, all the ^{32}P-5 S RNA was released from the complex (table 1, expt. 1). In the presence of 30 mM KCl, on the other hand, the Mg^{2+} concentration could be decreased to 0.1 mM without reduction of the 23 S RNA-bound radioactivity (table 1, expt. 2). Therefore, although the binding of ^{32}P-5 S RNA to 23 S RNA and fraction I proteins is not as stable as the binding of 5 S RNA to normal 50 S subunits, the ribonucleoprotein complex under study is nevertheless fairly stable, since it can be isolated on sucrose gradients containing either 300 mM KCl and 5 mM $MgCl_2$ or 30 mM KCl and 0.1 mM $MgCl_2$.

After isolation on a sucrose density gradient, the 23 S RNA–5 S RNA ribonucleoprotein complex was analyzed by sodium dodecylsulfate-polyacrylamide gel electrophoresis (fig. 2B). The pattern of protein bands was compared with that of fraction I (fig. 2A) and with that of the 23 S peak isolated after incubation of 23 S RNA and fraction I proteins in the absence of 5 S RNA (fig. 2C). For the sake of clarity, the bands in the pattern of fraction I have been arbitrarily numbered from 1 to 8.

The behaviour of the proteins corresponding to bands 2, 6, 7 and 8 was not influenced by the presence (fig. 2B) or absence (fig. 2C) of 5 S RNA in the incubation mixture. Proteins in band 2 were found associated to 23 S RNA in both cases, while proteins corresponding to bands 6, 7 and 8 were never recovered from the 23 S peak. With regard to the proteins in bands 1, 3, 4 and 5, their association with 23 S RNA was dependent upon the presence of 5 S RNA in the incubation mixture.

Although at their present stage, our experiments do not enable us to establish the exact number of proteins, which directly participate in the binding of 5 S RNA to the 50 S subunit, they demonstrate that only a few of the total 50 S proteins are indeed involved.

Acknowledgements

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